



STATE OF MARYLAND

DHMH

Maryland Department of Health and Mental Hygiene

Larry Hogan, Governor - Boyd Rutherford, Lt. Governor - Van Mitchell, Secretary

Laboratories Administration
Robert A. Myers, Ph.D., Director

DATE: February 12, 2016

TO: Medical Laboratory Directors, Local Health Officers, and Health Care Providers

FROM: Robert A. Myers, Ph.D. *RAM*
Director, Laboratories Administration

Maria Paz Carlos, Ph.D. *MC*
Chief, Division of Virology and Immunology, Laboratories Administration

RE: Updated Interim Guidance and Instructions for Submission of Specimens for Suspected Zika Virus Infection Testing at the Maryland DHMH Laboratory (February 12, 2016)

Effective immediately, the Maryland DHMH Laboratory will begin to perform Zika virus testing using both a reverse transcriptase polymerase chain reaction (RT-PCR) to detect viral RNA in acute specimens and an immunoglobulin (Ig) M enzyme-linked immunosorbent assay (ELISA) to screen specimens for the presence of Zika virus antibodies.

The MD DHMH Laboratory will also continue to test acute phase specimens approved for Zika virus testing for Dengue and Chikungunya viruses because these mosquito-borne viruses co-circulate in the same geographic areas and the infections can be difficult to distinguish clinically. Only Zika virus ELISA testing will be performed as an initial screen of specimens from pregnant women, who did not have symptoms for Zika virus infection, as per CDC's most recent guidance (Revised Diagnostic Testing for Zika, Chikungunya, and Dengue Viruses in US Public Health Laboratories February 7, 2016; <http://www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf>).

An infectious disease consultation by a DHMH or local health department (LHD) epidemiologist is still required to approve specimens for Zika Virus testing at the MD DHMH Laboratory prior to submission. This consultation will determine if the suspect case meets the clinical and travel criteria that would qualify the patient for testing. Contact the DHMH Infectious Disease Epidemiology and Outbreak Response Bureau at (410) 767-6700 (or after hours, at (410) 795-7365) or your LHD to arrange for consultation with an epidemiologist. Prior to contacting the DHMH or LHD epidemiologist, a review of the current interim CDC guidance found in the link below, is highly recommended.

<http://phpa.dhmh.maryland.gov/pages/zika.aspx>

Please note extensive cross-reactivity in flavivirus serological assays has been documented. Therefore, if specimens are reactive in the Zika IgM ELISA performed at the DHMH Laboratory, an additional paired convalescent serum might be required for submission to the CDC Laboratory for additional plaque reduction neutralization testing (PRNT) to possibly identify the most recently infecting flavivirus.

TEST REQUEST FORM

Complete the DHMH Laboratories Serological Testing Request Form No. 4677 when submitting specimens with prior approval for Zika virus testing to the DHMH Laboratory. Specimens submitted without prior approval from the Health Department will NOT be accepted for testing. Detailed instructions for collecting and submitting acceptable specimens, refer to the attached instructions on the DHMH Serological Testing Request Form No. 4677 Sample Form (Travel-Associated Zika Viral Infections Instructions for Specimen Submission February 12, 2016) or go to the MD DHMH Laboratories website (<http://dhmh.maryland.gov/laboratories>). **Please ensure that all required core demographic and contact information are completed.** In addition, please provide the following information to facilitate testing. Failure to include the additional clinical and epidemiological information might result in delays processing of specimens for testing.

- a. **Name of Health Department Person Approving Testing:** Please record on the requisition the name of the DHMH or local health department person approving the testing.
- b. **Clinical Illness/Compatible clinical presentation:** e.g., rash, acute onset fever, conjunctivitis, arthralgia
- c. **Pertinent travel history:** Recent travel to a region where local transmission of Zika virus has been documented (an updated list is available at <http://www.cdc.gov/zika/geo/index.html>)
- d. **History of any previous flavivirus infection:** e.g., West Nile virus (WNV), dengue virus
- e. **Acute illness on-set date:** contemporaneous with the travel exposures in areas of ongoing transmission (illness on-set date ≤ 14 days after exposure)
- f. **Immunization history:** Yellow fever (YF), Japanese encephalitis (JE), or Tick-borne encephalitis (TE) vaccines

SPECIMEN TYPES

Blood:

For antibody testing (ELISA IgM), 6-10 ml whole blood (red-top tube or serum separator tube) or 3-5 ml sera are acceptable. ELISA IgM testing will be performed on specimens collected ≥ 4 days after the on-set of illness.

For molecular testing (RT-PCR), in addition to serum (or whole blood), collect 6-10 whole blood in an EDTA purple-top tube or 3-5 ml plasma. Do NOT submit heparinized plasma (green-topped tubes). Heparin will inhibit PCR reactions. RT-PCR testing will be performed on acceptable blood specimens collected within 7 days of the onset of illness.

An additional convalescent specimen for IgM testing could be required to rule-out infections if the acute phase specimen is negative by both PCR and IgM testing after consultation with the health department. Convalescent serum might also be required for additional PRNT testing.

Properly package and transport to the lab on cold packs with completed DHMH Laboratories Serological Testing Request Form No. 4677 (see attached). (Keep specimens refrigerated until transported).

If more than 72 hours will pass before transporting the specimen to MD DHMH Laboratories, serum and plasma should be separated from whole blood and frozen. The serum and plasma should then be shipped frozen.

Urine:

It has been reported in the scientific literature that Zika virus RNA might persist longer in urine than in serum or plasma during the acute phase of the infection. According the MD DHMH Laboratory will accept urine **collected within 21 days from onset of illness for RT-PCR testing. Urine specimens must be submitted with whole blood/serum and EDTA whole blood/plasma specimens collected on the same date.** Urine specimens received without an accompanying serum and plasma specimens will not be tested. The results of urine testing will be reported for surveillance purposes in conjunction with the Zika RT-PCR results for a serum testing. If Zika virus RNA is only detected in the urine, follow-up Zika antibody testing of serum will be required to confirm seroconversion.

Collect 10-20 ml of urine in leak proof sterile plastic container without preservatives (e.g. urine cup). Label the container with the patient identifiers and the date of collection. Properly seal the container to prevent leaking and place the urine container in a separate sealable plastic bag. Submit the urine along with the serum specimen (see above) collected on the same date and transport to the lab on cold packs with completed DHMH Laboratories Serological Testing Request Form No. 4677 (see attached). (Keep specimens refrigerated until transported). Do not freeze the urine sample.

Other Specimen Types:

Detailed instructions on how to submit other specimen types (including amniotic fluid, cord blood, cerebrospinal fluid (CSF) and tissues) for Zika, dengue, chikungunya and other arboviral tests, contact the MD DHMH Laboratories at (443) 681-3923 or (443) 681-3937 during normal business hours from 8:00 a.m. - 4:30 p.m., Monday through Friday.

SHIPPING

Specimens collected from individuals for Zika virus testing may be transferred within the U.S. as Category B Biological substances in accordance with Department of Transportation (DoT) Hazardous Materials Regulations (49 CFR Part 171-180). Guidance for packaging samples in accordance with Category B Biological substance requirements can be found in the CDC/NIH Publication Biosafety in Microbiological and Biomedical Laboratories, 5th edition. Additional information on the DoT Hazardous Materials Transport Regulations can be found at <https://www.transportation.gov/bielines-hazmat>. Appropriately packaged specimens can be directly shipped via a public carrier to the MD DHMH Laboratory at the following address:

**Marvland DHMH Laboratories Administration
ATT: Arboviral Laboratory
1770 Ashland Avenue
Baltimore, MD 21205
(443) 681-3923 or (443) 681-3937**

Alternatively, contact your LHD for other available shipping arrangements to have specimens forwarded to the MD DHMH Laboratory.

Clinical laboratories currently performing chikungunya, dengue, or in the future plan to conduct Zika virus testing, are reminded to report cases of infections and submit clinical materials (i.e. serum, CSF, etc.) from these cases to the MD DHMH Laboratories as required by statute (Annotated Code of Maryland Health-General Article, §§18-201, 18-202, and 18-205, and Code of Maryland Regulations 10.06.01.03C: #9 Arboviral Infections).

Encl:

Updated Travel-Associated Zika Viral Infectious Instructions for Specimen Submission (February 12, 2016)
DHMH Laboratories Serological Testing Request Form No. 4677

cc: Dr. Howard Haft
Dr. David Blyth
Dr. Lucy Wilson
Dr. Richard Brooks
Dr. Katherine Feldman

Updated Travel-Associated Zika Viral Infections

Instructions for Specimen Submissions February 12, 2016

STATE LAB
Use Only

Laboratories Administration MD DHMH
1770 Ashland Ave. • Baltimore, MD 21205
443-681-3800 <http://dhhm.maryland.gov/laboratories/>
Robert A. Myers, Ph.D., Director

SEROLOGICAL TESTING

TYPE OR PRINT REQUIRED INFORMATION
PLACE LABELS ON ALL THREE COPIES

EH ☐ FP ☐ MYPIN ☐ NOD ☐ STD ☐ TB ☐ CD ☐ COR

Health Care Provider

Address

City

State

County

Zip Code

Contact Name:

Phone#

Fax#

Test Request Authorized by:

Sex: ☐ Male ☐ Female ☐ Transgender: M to F ☐ Transgender: F to M

Race: ☐ American Indian/Alaska Native ☐ Asian ☐ Black/African American ☐ Native Hawaiian/Other Pacific Islander

Ethnicity: Hispanic or Latino Origin? ☐ Yes ☐ No

Outbreak #

Submitter Lab #

Patent SS# (last 4 digits):

Last Name

First Name

Date of Birth (mm/dd/yyyy)

Address

City

State

Time Collected:

Date

Time

Previous Test Done? ☐ No ☐ Yes Name of Test:

Onset Date:

Exposure Date:

Vaccination History:

1st ☐ 2nd ☐ 3rd

1st ☐ 2nd ☐ 3rd

Clinical Illness/Symptoms

Must complete submitter information & include the name of the authorized person requesting the test.

Request Arbovirus Travel-Associated Panel, indicate "S" for Serum/whole clotted blood (ELISA), and "P" for plasma/whole blood EDTA un-clotted (PCR).

Complete patient's travel history (locations and dates), symptoms, vaccination history, & immune status.

For questions on Zika Virus testing, please contact the lab at: (443)681-3923/3937 during normal business hours from 8:00AM - 4:30PM, Monday-Friday.

For **ELISA IgM** testing, 6-10 ml whole blood (red-top tube or serum separator tube) or 3-5 ml sera are acceptable.

For **RT-PCR** testing, in addition to serum (or whole blood), collect 6-10 whole blood in an EDTA purple-top tube or 3-5 ml plasma. Do NOT submit heparinized plasma (green-topped tubes). Heparin will inhibit PCR reactions.

Transport to the lab on cold packs w/ completed Serological Testing Form. Separate serum & plasma then freeze if held >72 hours, & transport to the lab frozen.

If you are also submitting **URINE**, collect 10-20ml of urine in leak proof sterile plastic container w/o preservative (urine cup). Label urine cup with patient identifiers & date of collection. Submit urine w/ whole blood/serum. Urine ALONE will NOT be accepted.

Patient's first & last names must be on the specimen container & match exactly to the lab slip.

Collection date & onset date of symptoms fields must be completed.

Request CDC/Other Test(s), indicate "S" for Serum/whole clotted blood (ELISA), and "P" for plasma/whole blood EDTA un-clotted (PCR). If you are also submitting a specimen, indicate "U". Urine ALONE will NOT be accepted.

You MUST write both "Zika Virus" to request testing & the name of the DHMH/LHD Epidemiologist.

Arbovirus Panels (Serum or CSF)

Mandatory: Onset Date, Collection Date, and Travel History

Arbovirus Endemic Panel (WNV, EEE, SLE, LAC)

Arbovirus Travel-Associated Panel (Chikungunya, Dengue)

Based on information provided PCR and/or immunological assays will be performed.

Required information, check all that apply:

DIAGNOSIS: ☐ Aseptic Meningitis ☐ Encephalitis ☐ Other

SYMPTOMS: ☐ Headache ☐ Fever ☐ Stiff neck ☐ Altered mental state ☐ Muscle weakness ☐ Rash ☐ Other

ILLNESS FATAL? ☐ Yes ☐ No

TRAVEL HISTORY (dates and places)

IMMUNIZATIONS: Yellow fever? ☐ Yes ☐ No ☐ No ☐ No

IMMUNOCOMPROMISED? ☐ Yes ☐ No

Aspergillus

Chlamydia (group antigen IgG)

Cryptococcal (antigen)

Cytomegalovirus (CMV)

Ehrlichia

Epstein-Barr Virus (EBV)

Hepatitis A Screen (IgM Ab only, acute infection)

Call lab (443-681-3989) prior to submitting

Hepatitis B Screen (HBsAg, HBsAb)

Prenatal patient? ☐ Yes ☐ No

*Hepatitis B Panel: (HBsAg, HBsAb)

*Hepatitis B post vaccine (HBsAb)

Hepatitis C screen (HCV Ab only)

DHMH 4677 Revised 08/15

Specimen Source Code

Herpes Simplex Virus (HSV) Types 1 & 2

Legionella

Leptospira

Lyme Disease

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Mononucleosis - Infectious

Mumps Immunity Screen

Mycoplasma

Rocky Mountain Spotted Fever (RMSF)

*Rabies (RFFIT) (List vaccination dates above)

*Rubella Immunity Screen

*Rubella (Measles) Immunity Screen

Schistosoma

Strongyloides

Syphilis - Previously treated? ☐ Yes ☐ No

Toxoplasma

Tularemia

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Syphilis - Previously treated? ☐ Yes ☐ No

Toxoplasma

Tularemia

Varicella Immunity Screen

VDRL (CSF only)

Specimen Source Code

Herpes Simplex Virus (HSV) Types 1 & 2

Legionella

Leptospira

Lyme Disease

*MMRV Immunity Screen: (Measles (Rubella), Mumps, Rubella, Varicella (Chickenpox) IgG Ab only)

Mononucleosis - Infectious

Mumps Immunity Screen

Mycoplasma

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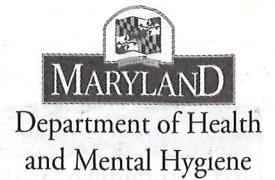
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**STATE LAB
Use Only**

Laboratories Administration MD DHMH
1770 Ashland Ave. • Baltimore, MD 21205
443-681-3800 <http://dhmh.maryland.gov/laboratories/>
Robert A. Myers, Ph.D., Director



SEROLOGICAL TESTING

TYPE OR PRINT REQUIRED INFORMATION OR PLACE LABELS ON ALL THREE COPIES	<input type="checkbox"/> EH <input type="checkbox"/> FP <input type="checkbox"/> MTY/PN <input type="checkbox"/> NOD <input type="checkbox"/> STD <input type="checkbox"/> TB <input type="checkbox"/> CD <input type="checkbox"/> COR			
	Health Care Provider		Patient SS# (last 4 digits):	
	Address		Last Name <input type="checkbox"/> SR <input type="checkbox"/> JR <input type="checkbox"/> Other _____	
	City	County	First Name	M.I.
	State	Zip Code	Date of Birth (mm/dd/yyyy)	/ /
	Contact Name:		Address	
	Phone#	Fax#	City	County
	Test Request Authorized by:		State	Zip Code
	Sex: <input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Transgender M to F <input type="checkbox"/> Transgender F to M		Ethnicity: Hispanic or Latino Origin? <input type="checkbox"/> yes <input type="checkbox"/> no	
	Race: <input type="checkbox"/> American Indian/Alaska Native <input type="checkbox"/> Asian <input type="checkbox"/> Black/African American <input type="checkbox"/> Native Hawaiian/other Pacific Islander <input type="checkbox"/> White			
	MRN/Case #	DOC #	Outbreak #	Submitter Lab #
	Date Collected:	Time Collected: <input type="checkbox"/> am <input type="checkbox"/> pm	*Vaccination History: _____	
	Previous Test Done? <input type="checkbox"/> no <input type="checkbox"/> yes		Name of Test _____ Date _____ <input type="checkbox"/> 1st <input type="checkbox"/> 2nd <input type="checkbox"/> 3rd State Lab Number: _____	
			Name of Test _____ Date _____ <input type="checkbox"/> 1st <input type="checkbox"/> 2nd <input type="checkbox"/> 3rd State Lab Number: _____	
	Onset Date: _____		Exposure Date: _____ <input type="checkbox"/> Clinical Illness/Symptoms: _____	

<p>↓ SPECIMEN SOURCE CODE</p> <p>Arbovirus Panels (Serum or CSF) Mandatory: Onset Date, Collection Date, and Travel History</p> <p>Arbovirus Endemic Panel (WNV, EEE, SLE, LAC)</p> <p>Arbovirus Travel-Associated Panel (Chikungunya, Dengue)</p> <p>Based on information provided PCR and/or immunological assays will be performed.</p> <p>Required information, check all that apply: DIAGNOSIS: <input type="checkbox"/> Aseptic Meningitis <input type="checkbox"/> Encephalitis <input type="checkbox"/> other _____</p> <p>SYMPTOMS: <input type="checkbox"/> headache <input type="checkbox"/> fever <input type="checkbox"/> stiff neck <input type="checkbox"/> altered mental state <input type="checkbox"/> muscle weakness <input type="checkbox"/> rash <input type="checkbox"/> other _____</p> <p>ILLNESS FATAL? <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>TRAVEL HISTORY (dates and places)</p> <p>IMMUNIZATIONS: Yellow fever? <input type="checkbox"/> yes <input type="checkbox"/> no Flavivirus? <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>IMMUNOCOMPROMISED? <input type="checkbox"/> yes <input type="checkbox"/> no</p>	<p>↓ SPECIMEN SOURCE CODE</p> <p>Herpes Simplex Virus (HSV) Types 1&2</p> <p>Legionella</p> <p>Leptospira</p> <p>Lyme Disease</p> <p>*MMRV Immunity Screen: [Measles (Rubeola), Mumps, Rubella, Varicella (Chickenpox) IgG Ab only]</p> <p>Mononucleosis - Infectious</p> <p>*Mumps Immunity Screen</p> <p>Mycoplasma</p> <p>Rocky Mountain Spotted Fever (RMSF)</p> <p>*Rabies (RFFIT) (*List vaccination dates above)</p> <p>*Rubella Immunity Screen</p> <p>*Rubeola (Measles) Immunity Screen</p> <p>Schistosoma</p> <p>Strongyloides</p> <p>Syphilis - Previously treated? <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>Toxoplasma</p> <p>Tularemia</p> <p>Varicella Immunity Screen</p> <p>VDRL (CSF only)</p> <p>CDC/Other Test(s)</p> <p>Add'l Specimen Codes _____</p> <p>Prior arrangements have been made with the following DHMH Labs Administration employee: _____</p> <p>_____</p> <p>_____</p> <p>_____</p> <p>Please Note Vaccination History above*</p>	<p>↓ SPECIMEN SOURCE CODE</p> <p>↓ LAVENDER TOP TUBE REQUIRED</p> <p>Hemoglobin Disorders</p> <p>Blood transfusion? (last 4 months) <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>Prenatal screen? <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>Father of baby screen? <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>Guardian's name if patient is a minor: _____</p> <p>Name of mother of "at risk" baby: _____</p> <p>RESTRICTED TEST Pre-Approved Submitters Only Submit a separate specimen for HIV Instructions go to: http://dhmh.maryland.gov/laboratories/</p> <p>HIV</p> <p>Country of Origin _____</p> <p>Rapid Test: <input type="checkbox"/> Reactive <input type="checkbox"/> Negative</p> <p>Date: _____</p> <p>Specimen stored refrigerated (2°-8°c) after collection. <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>Specimen transported on cold packs <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>SPECIMEN SOURCE CODE: PLACE CODE IN BOX NEXT TO TEST</p> <p>B Blood (5 ml)</p> <p>CSF Cerebrospinal Fluid</p> <p>L Lavender Top Tube</p> <p>P Plasma</p> <p>S Serum (1 ml per test)</p> <p>UR Urine</p>
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